3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

As discussed in the introduction, the mixture of atrazine, deethylatrazine, simazine, diazinon, and nitrate was chosen as the subject for this interaction profile on the basis of an analysis of the most frequently occurring mixtures in rural domestic and public water-supply wells (Squillace et al. 2002). The exposure scenario of greatest concern for this mixture is intermediate- to chronic-duration low-level oral exposure.

No adequate epidemiological or toxicological studies and no PBPK models are available for this mixture. Recommendations for exposure-based screening for the potential health hazard of this mixture are based on ATSDR (2001a) guidance, and comprise a components-based approach. This approach is used for the components with hazard quotients that equal or exceed 0.1, when at least two of the mixture components fulfill this criterion. Hazard quotients are the ratios of exposure estimates to noncancer health guidance values, such as MRLs. If only one or if none of the mixture components has a hazard quotient of this magnitude, no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. As discussed by ATSDR (1992, 2001a), the exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

Because there are sensitive reproductive endpoints in common to the triazine components of the mixture, the recommended approach (ATSDR 2001a) for atrazine/deethylatrazine and simazine is to estimate an endpoint-specific hazard index (by summing the hazard quotients for these components) for reproductive effects, using the guidance values shown in Table 6, or newer values as they become available. Hazard quotients are the ratios of exposures to MRLs, target-organ toxicity doses (TTDs), or other health guidance values. This process is shown in the following equation:

$$HI_{REPRO} = \frac{(E_{Atr}+E_{DEA})}{TTD_{Atr/DEA\ REPRO}} + \frac{E_{Smz}}{TTD_{Smz\ REPRO}}$$

where HI_{REPRO} is the hazard index for reproductive toxicity, E_{Atr} is the exposure to atrazine (as the oral intake in mg/kg/day), E_{DEA} is the exposure to deethylatrazine (as the oral intake in mg/kg/day), and $TTD_{Atr/DEA\ REPRO}$ is the TTD (in mg/kg/day) for the reproductive effects of oral exposure to atrazine and

deethylatrazine. Similarly, E_{Smz} is the exposure to simazine (as oral intake in mg/k/day) and $TTD_{Smz\ REPRO}$ is the TTD for the reproductive effects of oral exposure to simazine.

Table 6. MRLs and TTDs for Intermediate and Chronic Oral Exposure to Chemicals of Concern (See Appendices A, B, C, and D for Details)

Endpoint	Chemical			
	Atrazine[a]/ deethylatrazine (mg/kg/day)	Simazine[a] (mg/kg/day)	Diazinon (mg/kg/day)	Nitrate[a] (mg/kg/day)
Reproductive	0.0018[b]	0.0018[b]	NA	NA
Neurological	NA	NA	0.0002[c]	NA
Hematological	NA	NA	NA	1.6[d]

[a]The chemical interactions of atrazine and of simazine with nitrite (a metabolite of nitrate) produce N-nitrosoatrazine and N-nitrososimazine, but adequate toxicity data do not exist to characterize the endpoints of concern or to derive health guidance values for these nitrosamines. As discussed in the text, carcinogenicity is considered a likely endpoint of concern because most other N-nitrosamines are carcinogenic.
[b]Chronic dietary population adjusted dose (PAD) for atrazine and its chlorinated metabolites (combined) (EPA 2002b), adopted as target-organ toxicity dose (TTD) for atrazine and deethylatrazine (combined), and as an interim TTD for simazine.
[c]Intermediate oral MRL for diazinon.
[d]Chronic oral RfD for nitrate, adopted as TTD.

NA = not applicable

The weight-of-evidence analysis for interactions, summarized in the BINWOE determinations in Table 7, indicates that additivity is an appropriate assumption for the reproductive effects of atrazine/deethyl-atrazine and simazine, which act by a common mode of action on these endpoints, and can be considered dose additive. Confidence in the additivity assumption is high. The influence of diazinon and nitrate on the reproductive toxicity of these triazines, however, is indeterminate.

The neurological effects of diazinon are to be assessed with a separate hazard quotient for this chemical, because they are unique to the diazinon component of this mixture. This hazard quotient may underestimate the potential hazard of diazinon during co-exposure to atrazine, deethylatrazine, and simazine because the BINWOEs for the effects of these components on diazinon predict a greater-than-additive interaction (in this case, potentiation). Confidence in these predictions is medium. The influence of nitrate on the toxicity of diazinon is indeterminate.

Table 7. Matrix of BINWOE Determinations for Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Atrazine/ deethylatrazine	Simazine	Diazinon	Nitrate
EFFECT OF	Atrazine/ deethylatrazine		=IA (0) r	>IIB (+0.50) n	? (0) h >IIB (+0.50) c
	Simazine	=IA (0) r		>IIB (+0.50) n	? (0) h >IIB (+0.50) c
	Diazinon	? (0) r	? (0) r		? (0) h
	Nitrate	? (0) r >IIB (+0.50) c	?(0) r >IIB (+0.50) c	? (0) n	

r = reproductive, n = neurological, h = hematological, c = carcinogenic

The BINWOE determinations were explained in Section 2.3. No pertinent interactions data were available for the pairs of chemicals classified as indeterminate (?), and mechanistic information appeared inadequate, so indeterminate ratings were assigned to these pairs.

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a, 2001b):

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (–1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).

MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii: different route of exposure (0.79).

The hematological effects of nitrate also are to be assessed with a separate hazard quotient, because they are unique to the nitrate component of this mixture. The influence of the other mixture components on nitrate's hematological toxicity are indeterminate.

The potential carcinogenicity of the complete mixture is unknown. None of the individual components have been classified as carcinogenic (see Appendices), but atrazine and simazine can react with nitrite, the metabolite of nitrate, to form N-nitrosoatrazine and N-nitrososimazine. The potential carcinogenicity of these nitrosamines has not been investigated adequately. Genotoxicity studies indicate they are more genotoxic than the triazines and nitrate/nitrite from which they were formed. Confidence in greater-than-additive predictions for carcinogenicity are medium, as reflected in the BINWOEs, because of support from data on other N-nitrosamines and their precursors. Further exposure-based screening for cancer risk from N-nitrosoatrazine and N-nitrososimazine is not possible due to the lack of data regarding dose-response relationships for these compounds or their precursor mixtures.

If the hazard index for reproductive effects exceeds one, it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2001a). Similar preliminary conclusions apply if the hazard quotient for nitrate's hematological effects or diazinon's neurological effects exceeds one. The prediction that the triazines may potentiate the neurological toxicity of diazinon increases the concern, even at a diazinon hazard quotient slightly below one. If this screening procedure indicates preliminary evidence of a mixture health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2001a). This evaluation uses biomedical judgment, community-specific health outcome data, and consideration of community health concerns (ATSDR 1992) and the potential for carcinogenicity due to nitrosamine formation.